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Original Article

Effects of sodium hypochlorite on the viability and osteogenic differentiation of dental pulp stem cells in regenerative pulp therapy

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Abstract *Background/purpose:* Regenerative pulp therapy's efficacy is constrained by root canal disinfectants' effects on residual dental pulp stem cells. This study investigates sodium hypochlorite's influence on dental pulp stem cells (DPSCs) viability, mitochondrial function, and osteogenic differentiation, providing cellular and molecular foundations for optimizing clinical protocols.
Materials and methods: Human deciduous teeth-derived DPSCs were treated with sodium hypochlorite (0.5%–5%) for 1 min. Cell viability, cytoskeletal integrity, and mitochondrial morphology were assessed *via* CCK-8, immunofluorescence, and MitoTracker labeling. Osteogenic potential was evaluated through ALP activity, mineralized nodule quantification, and osteogenic marker expression. A juvenile rat model validated findings through immunohistochemistry and molecular analysis of periapical tissues.
Results: Sodium hypochlorite exhibited concentration-dependent effects. At $\leq$1%, DPSCs maintained >80% viability with intact cytoskeleton, while $\geq$3% significantly inhibited proliferation and induced mitochondrial autophagy. Low concentration (0.5%) minimally affected osteogenic differentiation markers, while higher concentrations progressively suppressed osteogenic potential. *In vivo* experiments corroborated these findings.

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Conclusion: Low-concentration sodium hypochlorite (≤0.5 %) preserves DPSC viability, mitochondrial function, and differentiation capacity, whereas higher concentrations impair cellular functions through autophagy induction. These findings support using lower concentrations in regenerative endodontics to maintain stem cell potential while ensuring adequate disinfection.

Introduction

Pulp regeneration, as a significant research direction in endodontics, aims to restore pulp vitality and promote root apex development, thereby extending tooth preservation duration.[1–4] Unlike traditional root canal therapy that focuses on eliminating infection and filling the canal, regenerative pulp therapy strives to reconstruct functional pulp–dentin complex, with the core concept being preservation of stem cell function within pulp tissue.[5–7] However, in clinical practice, the success rate and predictability of regenerative pulp therapy still face challenges, among which the impact of root canal disinfection processes on residual dental pulp stem cell viability and function remains a pressing scientific question.

Sodium hypochlorite (NaOCl), due to its broad-spectrum antimicrobial action and organic tissue dissolution capability, has become the preferred agent for root canal disinfection in regenerative pulp therapy.[8,9] The concentration of sodium hypochlorite used in clinical practice varies considerably (0.5%–5.25%), reflecting the difficulty in balancing effective disinfection and protection of pulp tissue. On one hand, high-concentration sodium hypochlorite provides stronger disinfection effects; on the other hand, it may damage stem cells in residual pulp tissue, thereby affecting tissue regenerative capacity.[10–13] This clinical contradiction constitutes the core scientific question of this study: how to ensure disinfection efficacy while maximally preserving the viability and differentiation potential of dental pulp stem cells?

Dental pulp stem cells (DPSCs), as a class of mesenchymal stem cells with self-renewal and multi-directional differentiation potential, play a crucial role in pulp tissue repair and regeneration.[14–17] DPSCs can differentiate into odontoblasts, osteoblasts, and other cell types, participating in dental hard tissue formation and pulp tissue regeneration.[18,19] Recent studies have shown that mitochondrial function is closely related to stem cell fate.[20–23] Mitochondria, as the center of cellular energy metabolism, not only affect cell survival but also directly participate in the regulation of stem cell self-renewal and differentiation.[24–27] Oxidative stress-induced mitochondrial damage may affect stem cell function through activation of mitophagy pathways.[28–30] Sodium hypochlorite, as a strong oxidizing agent, may regulate the viability and differentiation capacity of dental pulp stem cells by affecting mitochondrial function, but this mechanism has not been thoroughly explored in pulp regeneration research.

Deciduous dental pulp stem cells, due to their strong proliferative capacity, high differentiation potential, and easy acquisition, are considered an ideal model for pulp regeneration research.[31,32] Compared to adult dental pulp stem cells, deciduous dental pulp stem cells exhibit higher proliferation rates, stronger multi-directional differentiation abilities, and lower immunogenicity,[33] making them not only applicable to deciduous pulp regeneration research but also providing important references for permanent tooth regenerative pulp therapy.

Therefore, this study systematically explores the effects of different concentrations of sodium hypochlorite on deciduous dental pulp stem cell viability, mitochondrial function, and osteogenic differentiation capacity through a combination of *in vitro* and *in vivo* experiments. This research will provide scientific basis for clinical optimization of root canal disinfection strategies in regenerative pulp therapy, promoting the development of pulp tissue regeneration techniques.

Materials and methods

Isolation and culture of dental pulp stem cells

Dental pulp stem cells were isolated from healthy deciduous molars extracted due to normal exfoliation. Teeth were immediately preserved in 4 °C sterile saline after extraction. Under sterile conditions, pulp tissue was scraped from the pulp chamber using a 26G × $^1/_2$ inch injection needle. Tissue fragments were transferred to 35 mm culture dishes containing a specific culture medium system, including equal parts of F-12 Coon's and Ambesi's modified medium with 1.25% human serum (HS), Medium-199, and CMRL-1066, supplemented with key growth factors (Sigma–Aldrich, St. Louis, MO, USA). Cells were cultured at 37 °C in 5% CO_2, with the culture dish left undisturbed for the initial 3 days to ensure tissue attachment. Cell surface marker expression was confirmed by flow cytometry to verify dental pulp stem cell characteristics. Experiments utilized passages 3–5 cells, which exhibited typical fibroblast-like morphology and good proliferative capacity.[34]

Effect of sodium hypochlorite on dental pulp stem cell viability

We first evaluated the effect of different concentrations of sodium hypochlorite on dental pulp stem cell viability. DPSCs were seeded in 96-well plates at a density of 5 × 10^3 cells/well, cultured for 24 h, then treated with 0.5%, 1%, 3%, and 5% concentrations of sodium hypochlorite (Clorox Company, Oakland, CA, USA) for 1 min, followed by

PBS washing and continued culture. Cell viability was detected by CCK-8 assay (Dojindo Molecular Technologies, Kumamoto, Japan) on days 1, 3, and 5.[35]

Effect of sodium hypochlorite on dental pulp stem cell cytoskeleton

We used immunofluorescence staining to observe cytoskeletal structure (FITC-labeled phalloidin staining (Thermo Fisher Scientific, Waltham, MA, USA)) and cell nuclei (DAPI staining (Thermo Fisher Scientific)). Fluorescence images were acquired through laser confocal microscopy to assess cytoskeletal integrity.

Effect of sodium hypochlorite on dental pulp stem cell mitochondria

We used immunofluorescence staining to observe mitochondrial morphology (MitoTracker-labeled mitochondria (Thermo Fisher Scientific)) and cell nuclei (DAPI staining). Fluorescence images were acquired through laser confocal microscopy to assess mitochondrial morphology.

Effect of sodium hypochlorite on dental pulp stem cell osteogenic differentiation

We selected appropriate concentrations of sodium hypochlorite to pretreat dental pulp stem cells, then placed the cells in osteogenic induction medium (containing β-glycerophosphate sodium, retinoic acid, dexamethasone, etc. (Sigma–Aldrich)). Osteogenic differentiation capacity was evaluated through multiple methods.[36]

Morphological observation

Cell morphological changes and cytoskeletal reorganization were observed on day 7 of osteogenic induction. FITC-labeled phalloidin was used to mark the cytoskeleton, and DAPI to label cell nuclei.

ALP staining and activity detection

After 14 days of osteogenic induction, alkaline phosphatase (ALP) staining (Sigma–Aldrich) was performed, ALP activity was detected and standardized to protein content (U/g protein).

Mineralized nodule formation

After 21 days of osteogenic induction, Alizarin Red S (ARS) staining (Sigma–Aldrich) was performed to detect mineralized nodule formation, with quantitative analysis by spectrophotometry.

Osteogenic-related gene expression

Real-time fluorescence quantitative PCR was used to detect mRNA expression levels of key osteogenic regulatory factors (Runx2, ALP, and OCN) at different time points (7, 14, and 21 days).

Protein expression analysis

ELISA (R&D Systems, Minneapolis, MN, USA) was used to analyze protein expression levels of the aforementioned osteogenic markers. All *in vitro* experiments were independently repeated at least three times to ensure reliability and reproducibility of results.

Juvenile rat deciduous tooth root canal treatment model

This study used 4-week-old SPF-grade male SD rats (weight 80 ± 10g), purchased from Dashuo Experimental Animal Center (Chengdu, China). All animal experiments were approved by the Experimental Animal Ethics Committee of Sichuan University (Approval No. WCHSIRB-D-2022-280). Animals were housed in animal rooms with controlled temperature (22 ± 2 °C), humidity (55 ± 5%), and 12-h light/dark cycles, with free access to food and water. We established a juvenile rat deciduous tooth root canal treatment model. Rats were randomly divided into 5 groups (n = 6/group): control group (saline), 0.5% sodium hypochlorite group, 1% sodium hypochlorite group, 3% sodium hypochlorite group, and 5% sodium hypochlorite group. Root canal treatment procedures were performed under anesthesia: pulp exposure, root canal preparation, irrigation with different concentrations of sodium hypochlorite (1 min), saline irrigation, and temporary coronal sealing.

Pathological staining analysis

Rats were sacrificed 2 weeks after treatment, and periapical tissue samples from treated teeth were collected for analysis. Samples were fixed, decalcified, embedded, and sectioned for testing. Immunohistochemical staining methods were used to detect ALP expression in periapical tissues. Immunofluorescence staining methods were used to detect the expression and distribution patterns of OCN (red fluorescence) and OPN (green fluorescence), with cell nuclei counterstained with DAPI (blue).

Gene and protein expression analysis

RNA and protein were extracted from treated periapical tissues to detect gene and protein expression levels of ALP, OCN, and OPN. Real-time fluorescence quantitative PCR was used to detect mRNA expression levels of key osteogenic regulatory factors (ALP, OCN, and OPN). ELISA (R&D Systems) was used to analyze protein expression levels of the aforementioned osteogenic markers, standardized to GAPDH as an internal reference. All *in vitro* experiments were independently repeated at least three times to ensure reliability and reproducibility of results.

Statistical analysis

All data were statistically analyzed using SPSS 25.0 software, with results expressed as mean ± standard deviation ($\bar{x} \pm s$). Data normality and variance homogeneity were first tested, and data meeting these conditions were analyzed

using one-way analysis of variance (ANOVA) and Bonferroni-corrected multiple comparisons. $P < 0.05$ was considered statistically significant.

Results

Effect of sodium hypochlorite on dental pulp stem cell viability

To assess the effect of sodium hypochlorite on dental pulp stem cell viability, we first observed morphological changes in the cell cytoskeleton after treatment with different concentrations of sodium hypochlorite through immunofluorescence staining. As shown in Fig. 1A, in the control group and 0.5% sodium hypochlorite group, cell actin fibers (green) were arranged regularly, showing typical spindle-shaped structures, with intact cell nuclei (blue); the 1% sodium hypochlorite group maintained a similar cell morphology; while the 3% and 5% groups showed a notable reduction in cell density compared to lower concentration groups. CCK-8 detection results indicated that sodium hypochlorite's effect on DPSCs viability showed clear concentration dependence (Fig. 1B—D). On day 1 after treatment (Fig. 1B), there was no statistically significant difference in cell viability among the 0.5 % group, 1 % group, and control group ($P > 0.05$); while cell viability in the 3% and 5% groups was significantly reduced ($P < 0.05$). Results from day 3 (Fig. 1D) and day 5 (Fig. 1C) showed that low concentration groups (0.5% and 1%) had small differences from the control group, still within acceptable ranges; while high concentration groups (3% and 5%) showed further increased differences in cell viability compared to the control group.

Overall, low concentration treatments had relatively limited effects on cytoskeletal structure and cell viability, with no significant differences compared to the control group, indicating that within this concentration range, DPSCs maintained good biological function and proliferative capacity. High concentration treatments significantly

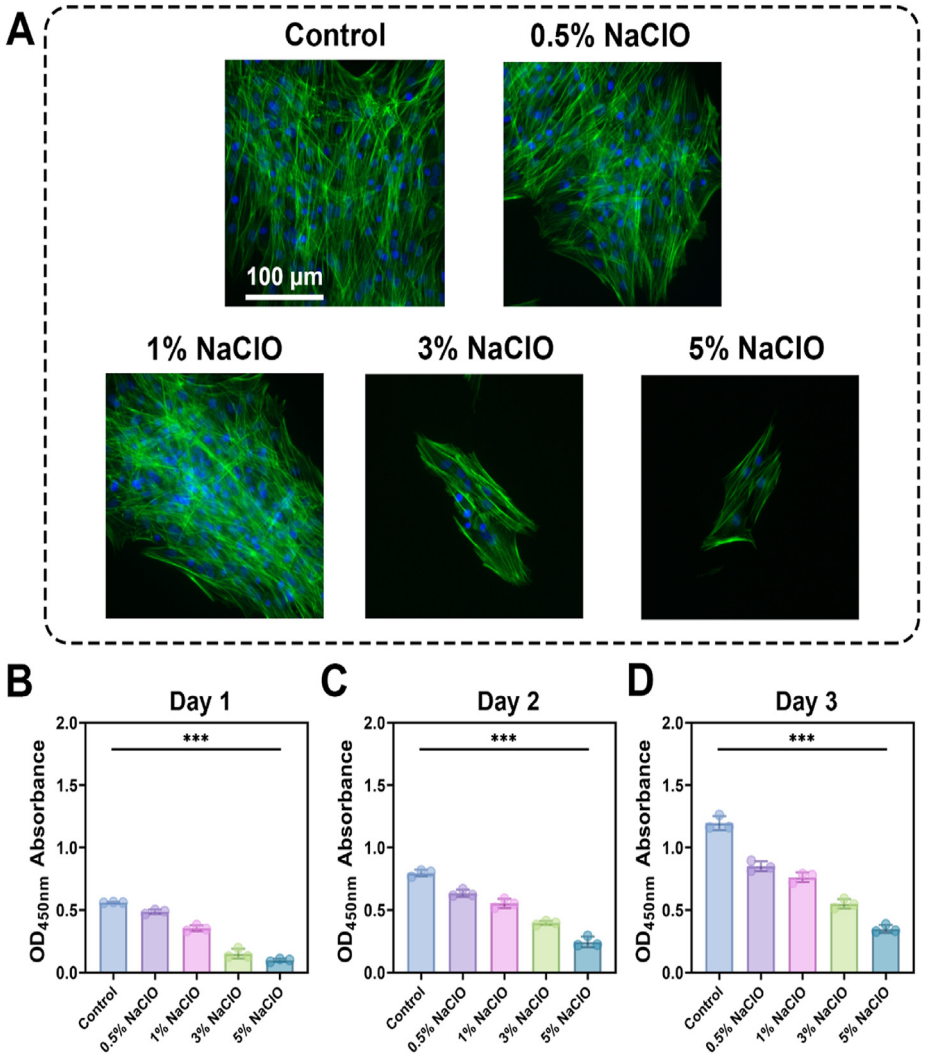

Figure 1 Effects of sodium hypochlorite on DPSC viability. (A) Immunofluorescence staining of DPSC cytoskeleton (green: actin filaments; blue: nuclei) (NaClO: sodium hypochlorite). **(B)** CCK-8 assay results on day 1, **(C)** day 5, and **(D)** day 3 after treatment with different concentrations of sodium hypochlorite.

reduced cell viability, and this inhibitory effect was further intensified with extended time.

Effect of sodium hypochlorite on dental pulp stem cell mitochondrial function

Since mitochondria play important roles in cell metabolism, proliferation, and differentiation, we further explored the effect of sodium hypochlorite on DPSCs mitochondrial function. As shown in Fig. 2A, we proposed the hypothesis that sodium hypochlorite might affect DPSCs viability by inducing mitochondrial autophagy. To verify this hypothesis, we used MitoTracker to label cell mitochondria (Fig. 2B), revealing that mitochondria in the control group exhibited typical tubular and rod-like structures, evenly distributed throughout the cytoplasm; the 1% group showed partially fragmented mitochondrial networks with only a few punctate structures; while the 3% group had severely fragmented mitochondria with diffuse punctate distribution, indicating significantly reduced mitochondrial quality.

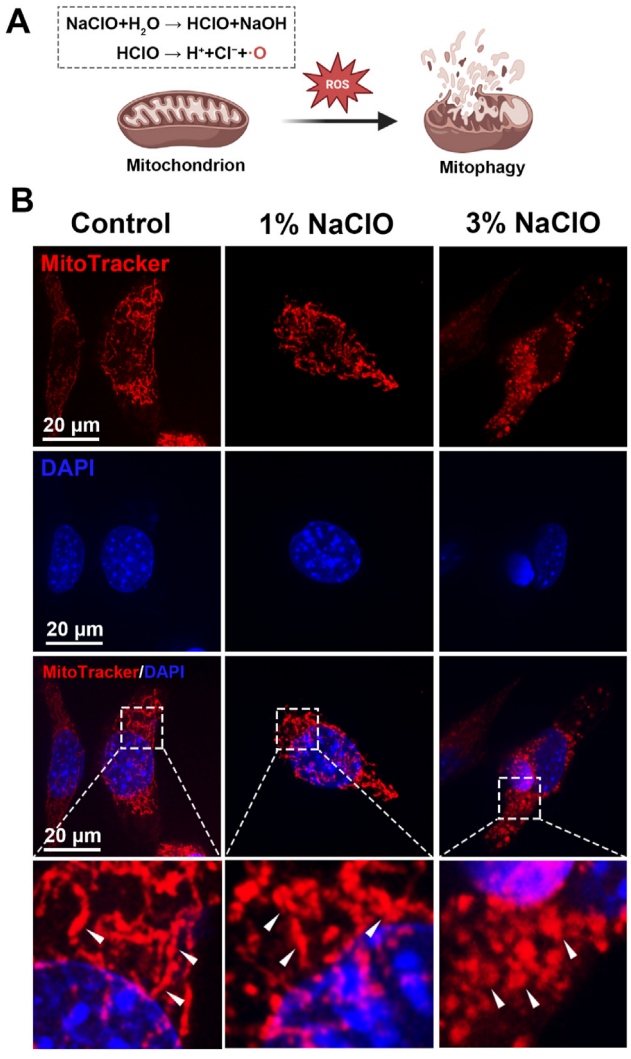

Figure 2 Influence of sodium hypochlorite on mitochondrial function in DPSCs. (A) Schematic diagram illustrating sodium hypochlorite-induced mitochondrial autophagy. **(B)** Mitochondrial morphology visualized by MitoTracker labeling. (red: mitochondria; blue: nuclei).

These results suggest that high concentrations of sodium hypochlorite (≥3%) may affect DPSCs viability through inducing mitochondrial damage and autophagy, while low concentrations (0.5%–1%) have minimal effects on mitochondrial morphology and function. Mitochondria play a central role in cellular energy metabolism and provide important energy support for the subsequent osteogenic differentiation of DPSC cells. Therefore, high-concentration sodium hypochlorite treatment is likely to severely affect the osteogenic differentiation of DPSC cells.

Effect of sodium hypochlorite on dental pulp stem cell osteogenic differentiation

Morphological and histochemical analysis

To assess the effect of sodium hypochlorite on DPSCs osteogenic differentiation capacity, we first observed changes in cell morphology and cytoskeletal structure during osteogenic induction. As shown in Fig. 3A, on day 7 of osteogenic induction, cells in the control group and 0.5% sodium hypochlorite group exhibited typical polygonal osteoblast-like morphology with intact, regularly arranged cytoskeletal structures (green); cells in the 1% group had more irregular morphology than the control group, with looser cytoskeletal arrangements; the 3% and 5% groups showed significantly reduced cell numbers with sparser cytoskeletal structures.

ALP staining results (Fig. 3B) showed that on day 14 of osteogenic induction, both the control group and 0.5% group exhibited obvious blue-purple ALP-positive areas with similar staining intensities; the 1% group showed significantly weaker staining intensity; the 3% and 5% groups showed almost no obvious positive staining. Quantitative analysis (Fig. 3D) showed that ALP activity in the 0.5% group (11.80 ± 1.27 U/g) was slightly lower than in the control group, but the difference was not statistically significant ($P > 0.05$); while activities in the 1% group (8.43 ± 0.97 U/g), 3% group (5.03 ± 0.66 U/g), and 5% group (3.08 ± 0.94 U/g) were significantly lower than in the control group ($P < 0.05$, $P < 0.01$, $P < 0.001$), showing a clear concentration-dependent downward trend.

Alizarin Red S staining (ARS) assessment of mineralized nodule formation (Fig. 3C) showed that on day 21 of osteogenic induction, the control group and 0.5% group formed numerous red mineralized nodules with uniform distribution; the 1% group showed significantly reduced quantity and area of mineralized nodules; the 3% and 5% groups showed almost no large-area mineralized nodule formation. Quantitative analysis results (Fig. 3E) showed that compared to the control group, mineralized nodule formation in the 0.5% group was 78.85% ± 7.21% of the control group, showing a decrease but with no significant difference ($P > 0.05$); the 1% group was 48.56% ± 5.32% of the control group, significantly decreased ($P < 0.01$); and the 3% and 5% groups were only 19.23% ± 1.97% and 12.50% ± 2.43% of the control group, respectively. These results further confirm that low concentrations of sodium hypochlorite (0.5%–1%) have limited effects on DPSCs' osteogenic differentiation capacity, while medium to high

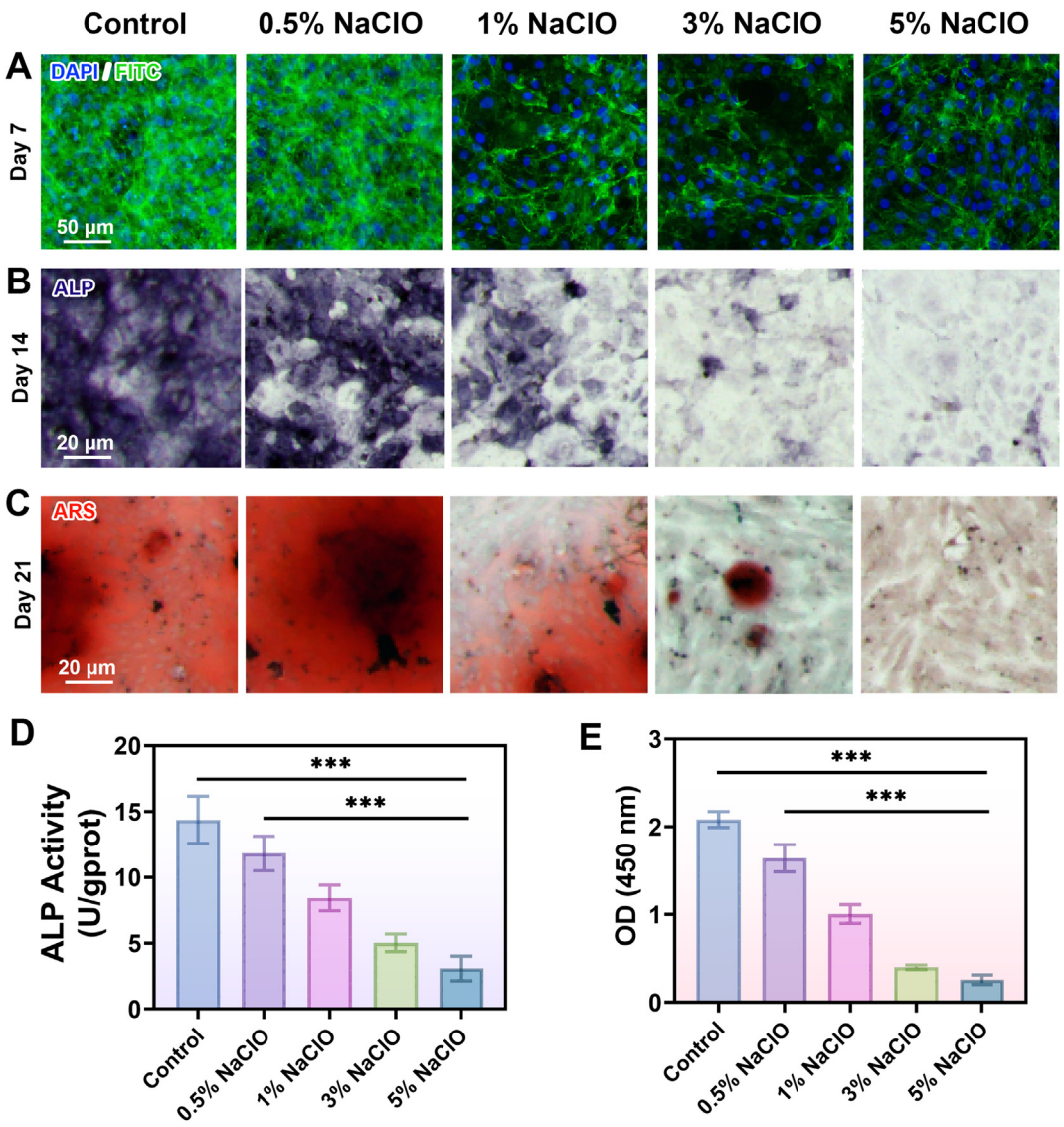

Figure 3 Effects of sodium hypochlorite on osteogenic differentiation capacity of DPSCs. (A) Cytoskeletal staining on day 7 of osteogenic induction (green: cytoskeleton; blue: nuclei). **(B)** Alkaline phosphatase (ALP) staining on day 14 of osteogenic induction. **(C)** Alizarin red S (ARS) staining on day 21 of osteogenic induction. **(D)** Quantitative analysis of ALP activity. **(E)** Quantitative analysis of mineralized nodule formation by ARS staining.

concentrations ($\geq$3%) significantly inhibit DPSCs' osteogenic differentiation potential, with this inhibitory effect strengthening as concentration increases, showing a clear dose-dependent relationship.

Osteogenic-related gene and protein expression

To further clarify the effect of sodium hypochlorite on DPSCs osteogenic differentiation capacity, we detected gene and protein expression levels of key osteogenic regulatory factors Runx2, ALP, and OCN (Fig. 4). During osteogenic induction, compared to the control group, the 0.5% sodium hypochlorite group showed no significant differences in gene expression of Runx2, ALP, and OCN. The 1% group showed slightly reduced expression levels of all three genes; while the 3% and 5% groups showed significantly lower expression levels than the control group at all time points. ELISA protein quantitative detection results showed high consistency with gene expression patterns.

The above data reveal the concentration-dependent characteristics of sodium hypochlorite's effect on DPSCs osteogenic differentiation capacity. Low concentration (0.5%) sodium hypochlorite had relatively limited effects on DPSCs osteogenic-related gene and protein expression, with no significant statistical differences compared to the control group; the 1% concentration treatment group began to show significant inhibitory effects; while concentrations of 3% and above significantly inhibited the expression of key molecular regulatory factors in the osteogenic differentiation process, with this inhibition degree intensifying as concentration increased, indicating that sodium hypochlorite affects DPSCs' osteogenic differentiation potential by interfering with key molecules in osteogenic differentiation signaling pathways.

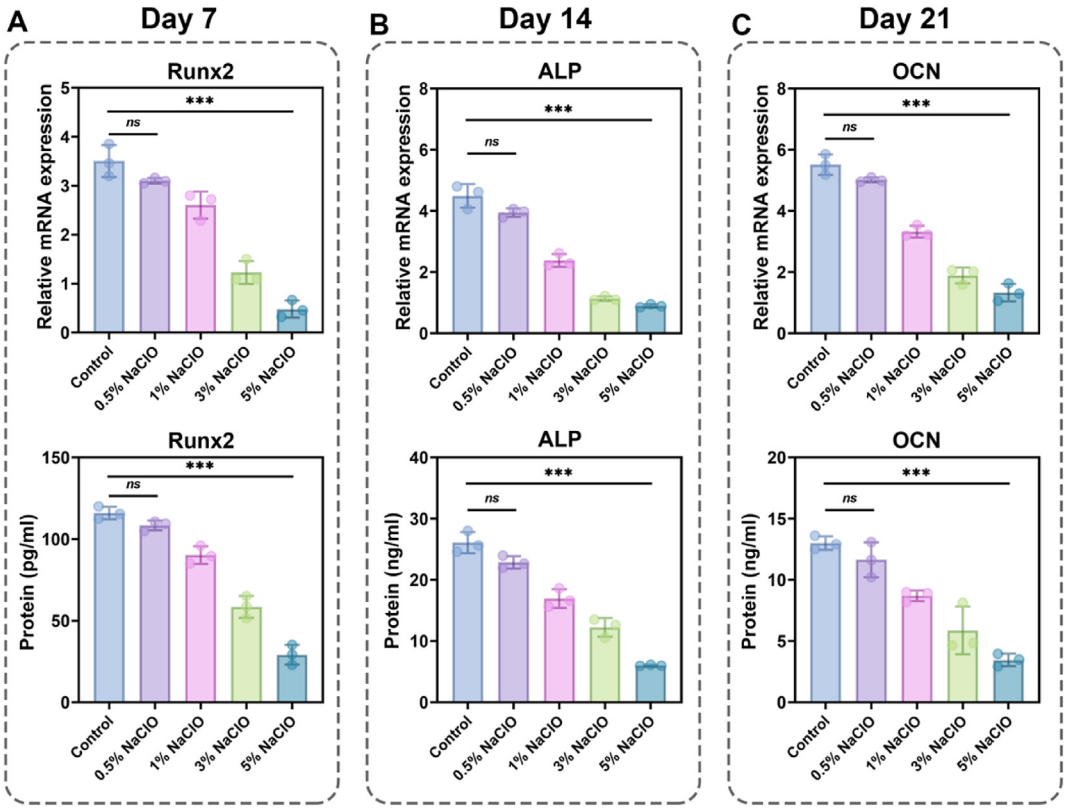

Figure 4 Molecular assessment of osteogenic differentiation capacity in sodium hypochlorite-treated DPSCs. Gene and protein expression levels of **(A)** Runx2 (Runt-related transcription factor 2), **(B)** ALP (Alkaline Phosphatase), and **(C)** OCN (Osteocalcin) at different time points following treatment with varying concentrations of sodium hypochlorite.

In vivo experimental study of sodium hypochlorite's effect on DPSCs

Histopathological staining analysis

We established a juvenile rat deciduous tooth root canal treatment model to verify *in vitro* experimental results (Fig. 5A). After root canal treatment of rat mandibular first molars, immunohistochemical and immunofluorescence methods were used to detect the expression of osteogenic-related markers in periapical tissues. ALP immunohistochemical staining results (Fig. 5B) showed abundant ALP expression in pulp tissues of the control group and 0.5% sodium hypochlorite group; the 1% group showed weakened ALP expression with reduced positive cell numbers; the 3% and 5% groups showed almost no ALP-positive expression in pulp tissues.

OCN immunofluorescence staining results (Fig. 5C) were similar to ALP expression patterns, with abundant OCN expression in pulp tissues of the control group and 0.5% group; as sodium hypochlorite concentration increased, OCN-positive signals gradually weakened, almost completely disappearing in the 5% group. OPN immunofluorescence staining results (Fig. 5D) showed the same findings.

Osteogenic-related gene and protein expression *in vivo*

We further detected gene and protein expression of bone-related markers in periapical tissues after treatment (Fig. 6). Results showed that compared to the control group, gene expression levels of ALP, OCN, and OPN in the 0.5% sodium hypochlorite group were 90.8 ± 6.5%, 86.2 ± 5.8%, and 84.5 ± 6.3% of the control group, respectively ($P > 0.05$); the 1% group decreased to 71.6 ± 7.2%, 74.3 ± 5.9%, and 72.5 ± 7.8% of the control group, respectively ($P < 0.05$); the 3% and 5% groups both significantly decreased to 30–50% of the control group ($P < 0.01$).

ELISA detection showed that protein expression levels were consistent with gene expression patterns, with the 0.5% group similar to the control group, while the 1%, 3%, and 5% groups showed concentration-dependent decreases. These results indicate that *in vivo* experiments again verified that 0.5% sodium hypochlorite is a relatively safe concentration, capable of providing root canal disinfection effects without significantly affecting the expression of bone-related markers in pulp tissue.

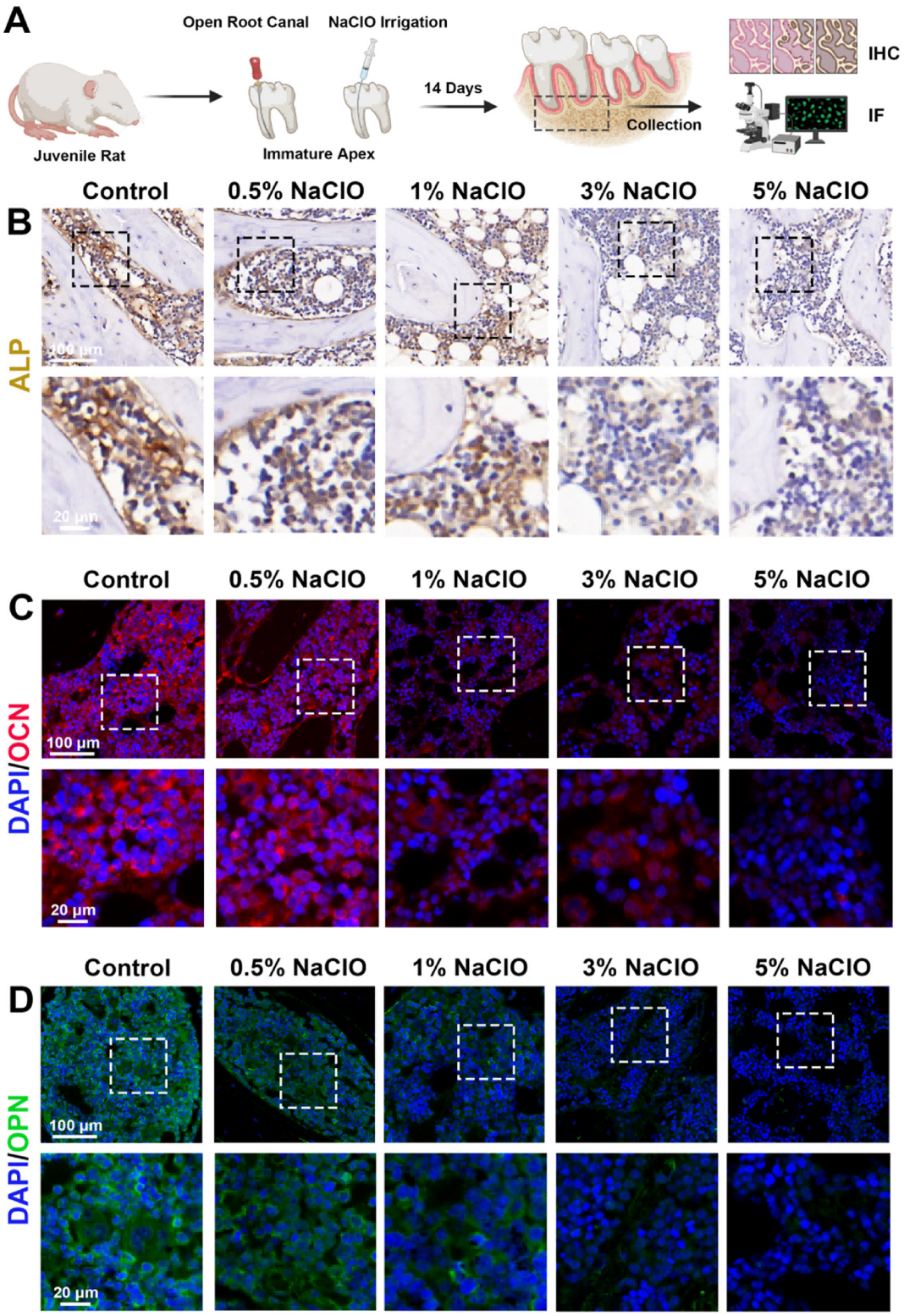

Figure 5 ***In vivo*** **juvenile rat model study. (A)** Experimental protocol workflow. **(B)** Immunohistochemical staining of ALP (Alkaline Phosphatase) expression. **(C)** Immunofluorescence staining of OCN (Osteocalcin) (red: OCN; blue: nuclei). **(D)** Immunofluorescence staining of OPN (Osteopontin) (green: OPN; blue: nuclei).

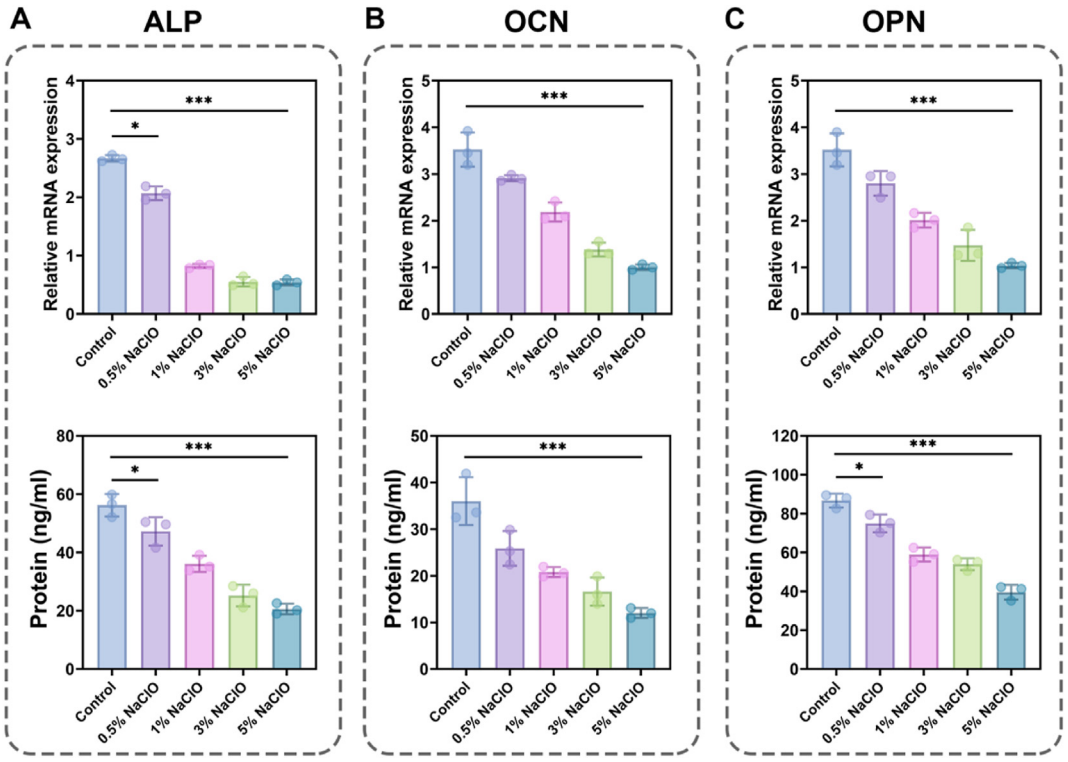

Figure 6 Molecular analysis of juvenile rat tissue samples. Gene and protein expression levels of **(A)** ALP (Alkaline Phosphatase), **(B)** OCN (Osteocalcin), and **(C)** OPN (Osteopontin) in periapical tissues following treatment with different concentrations of sodium hypochlorite.s

Discussion

The sodium hypochlorite treatment time (1 min) selected in this study approximates actual clinical operation time, enhancing the clinical relevance of the research findings. In clinical practice, there has been ongoing controversy regarding the selection of concentration and time for root canal disinfection.[37,38] Our research suggests that in regenerative pulp therapy, 0.5%–1% sodium hypochlorite may be a relatively ideal concentration, providing sufficient antimicrobial effects while maximally preserving the viability and differentiation potential of stem cells in residual pulp tissue and periapical tissues.

The molecular mechanisms underlying sodium hypochlorite-induced mitochondrial damage in dental pulp stem cells involve complex cellular stress responses and metabolic disruption. At high concentrations, sodium hypochlorite triggers oxidative stress through excessive ROS generation, which critically impairs mitochondrial membrane integrity and electron transport chain function. This oxidative assault leads to mitochondrial membrane potential collapse, cytochrome *c* release, and activation of intrinsic apoptotic signaling pathways. The mitochondrial dynamic equilibrium is fundamentally altered, shifting from a balanced fusion-fission process to a predominantly fragmented state through dysregulation of key mitochondrial fission proteins like Drp1 (dynamin-related protein 1) and mitochondrial fusion proteins such as Mfn2 (mitofusin 2). Moreover, sustained mitochondrial damage activates selective autophagy mechanisms, particularly mitophagy, as a cellular survival strategy to eliminate severely compromised mitochondria. This process is mediated through PINK1-Parkin pathway activation, which serves as a critical quality control mechanism to prevent widespread cellular dysfunction. The mitochondrial stress response ultimately triggers complex cellular signaling cascades, which further modulate cellular metabolism, proliferation, and differentiation potential of dental pulp stem cells.

Some limitations in this study need to be addressed in future research. First, we primarily focused on the direct effects of sodium hypochlorite on dental pulp stem cells, whereas in clinical practice, the interaction between sodium hypochlorite and dentin matrix may release bioactive molecules, thereby indirectly affecting dental pulp stem cell function. Second, although we explored the role of mitochondrial autophagy in sodium hypochlorite's effect on dental pulp stem cell viability, the specific molecular mechanisms still need further elucidation. Additionally, the effects of EDTA, chlorhexidine, and other agents commonly used in conjunction with sodium hypochlorite on dental pulp stem cells are also worth in-depth investigation.

Our study is the first to reveal the mechanism by which sodium hypochlorite regulates dental pulp stem cell viability by affecting mitochondrial function in the context of pulp regeneration. Mitochondria are the center of cellular energy metabolism and the primary target of oxidative stress reactions. High-concentration sodium hypochlorite ($\geq$3%) leads to mitochondrial network fragmentation and dysfunction, thereby activating mitochondrial autophagy processes.

This study systematically explored the effects of different concentrations of sodium hypochlorite on dental pulp stem cell viability, mitochondrial function, and osteogenic differentiation capacity through a combination of *in vitro* and *in vivo* experiments. The results show that low concentration (0.5%) sodium hypochlorite has minimal effects on dental pulp stem cell viability and osteogenic differentiation capacity, maintaining normal mitochondrial function; while high concentration sodium hypochlorite significantly inhibits stem cell function through damaging mitochondrial function and inducing autophagy. These findings provide scientific basis for clinical optimization of root canal disinfection strategies in regenerative pulp therapy, suggesting the use of lower concentration sodium hypochlorite to maintain dental pulp stem cell viability and differentiation potential while ensuring disinfection efficacy, thereby promoting pulp tissue regeneration.

Declaration of competing interest

The authors have no conflicts of interest relevant to this article.

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